be dependent upon claim 1, whereby claim 14 further defines the measuring step generically claimed in claim 1.

It is respectfully submitted that the above amendments are merely housekeeping amendments which simplify the issues by clarifying the characterizing aspects of the invention, deleting redundant claims, and potentially simplifying issues that may be on appeal, if necessary. The amendments raise no new issues nor require any further searching as the scope of the claims are essentially the same as previously set forth, only in a more exact and simplified manner.

The only basis for rejection of the claims set forth in the outstanding Office Action is a rejection under 35 U.S.C. § 103 holding that the claims are unpatentable over the Morrow et al. reference, a reference cited on page 4 of the originally filed specification by applicants. It is respectfully submitted that applicants set forth below a basis for two conclusions supporting patentability of the present invention. First, the Office Action fails to set forth any basis for modifying the teachings within the four corners of the Morrow et al. reference to derive the present invention. The only suggestion for modifying the Morrow et al. reference to derive the present invention is set forth in the specification of the presently pending application. Second, if it were to be admitted, arguendo, that the present Office Action sets forth a basis for a *prima facie* obviousness rejection, then applicants set forth herein a factual basis rebutting the *prima facie* obviousness rejection. The factual evidence

set forth below demonstrates that the present invention provides unexpected results not at all suggested to one skilled in the art.

1. The Morrow et al. reference discloses novel prostaglandins as artifacts and not as an indicator of any type of syndrome or stress.

The Morrow et al. reference generally discloses an analysis of fresh plasma from normal volunteers by GC/MS which reveal PGF_2 compounds. The levels of these compounds were markedly increased after several months of storage. By various approaches, it was found that these compounds in stored and base-treated plasma were in fact PGF_2 compounds. Most significant was the finding that the formation of these compounds were found to occur by a nonenzymatic oxidative process. The purpose of the investigation was to determine the processes involved leading to the detection of markedly elevated levels of the these putative PGF_2 compounds in plasma during storage.

The results of the analysis, as stated above, are that these prostaglandins were formed by a nonenzymatic oxidative process. As stated on page 8 of the paper, "[t]his nonenzymatic oxidative formation of PGF_2 compound appears to be a facile process which occurs very readily in biological fluids". As stated in the first column, first full paragraph, on page 9 of the paper, the authors caution that "special precautions clearly need to be taken to minimize nonenzymatic formation of prostaglandins with plasma and probably any biological sample containing phospholipids in which prostaglandins are being measured". Further precautions are set forth in the same paragraph wherein the authors state "the same

analytical concerns regarding nonenzymatic formation of prostaglandins also likely apply to *in vitro* studies involving incubation of cells and tissues". The second paragraph of the same column of the Morrow et al. reference, specifically cautions that "...nonenzymatic formation of protaglandins can yield misleading results which relates to the previous report of multiple isomeric PGF_2 compounds in plasma which were thought to arise from 11-ketoreductase metabolism of PGD_2". From the aforementioned quote, the paper concludes in the last sentence thereof, on page 10, that "[c]learly, these findings have important **analytical** ramifications regarding analysis of cyclooxygenase-derived $PGF_{2\ a}$, PGF_2 compounds derived from 11-ketoreductase metabolism of PGD_2, and potentially other prostaglandins and eicosanoids". In other words, within the four corner of the Morrow et al. reference, the results relate to the detection of potential artifacts done on the analysis of samples, and they caution that these artifacts must be recognized in the measurement of the various prostaglandins and eicosanoids compounds. The Morrow et al. reference does not disclose nor even suggest any significance for these artifact compounds relating to any syndrome, any stress, or that one should perform further analysis on the same in relation to any state. Rather, the Morrow et al. reference only suggests caution in analyzing samples.

The Office Action acknowledges that the Morrow et al. reference does not teach a method of determining oxidative stress *in vivo*. In fact, a strict reading of the Morrow et al. reference shows that the Morrow et al. reference does not relate to any method of determining oxidative stress. Rather, the Morrow et al. reference relates to the detection of artifacts which might effect the analysis of other prostaglandin compounds. The Office

Action further states that "it would be obvious to select and use any known method of interest for the intended purpose of determining oxidative stress *in vivo*." The issue is "where is the motivation, suggestion, etc. to do so outside of the presently pending application?" Absent some statement in the Office Action for a suggestion or motivation to modify the Morrow et al. teaching beyond what is stated in the Morrow et al. reference, then it must be concluded that there is no basis for a *prima facie* obviousness rejection. That is, there is absolutely no basis short of the presently pending specification or a conjecture made in the Office Action to modify the Morrow et al. reference.

It is black letter law that the reference cannot be modified through hind-sight after reviewing a pending application under prosecution or by conjecture. It is black letter law that the issue is not whether a principle reference can be modified on the basis of what is obvious to the Examiner, but is obvious to one skilled in the art. The determination to what is obvious to one skilled in the art must be based upon disclosures and prior art references.

The Office Action admits that that Morrow et al. reference is a primary reference which does not teach invention as set forth in the presently pending claims. Further, if one would be able to take any primary reference, and then begin to modify it by means outside of the reference holding that one of ordinary skill in the art could do so without having a reference demonstrating as same, then there would be no patentable inventions. Rather, as a matter of law, a primary reference can only be modified by suggestions within the reference to do so or suggestions in secondary references. The Office

Action is absolutely silent as to where there is any suggestion in the Morrow et al. reference for its own modification to derive the present invention. It is also absolutely silent with regard to the secondary references which would do the same. This is because the present invention is patentably distinguishable over the Morrow et al. reference.

In view of the above, it is respectfully submitted that there is absolutely no dispute that the Morrow et al. reference does not disclose the present invention. Moreover, there does not exist nor is there set forth in the presently pending Office Action any reference to any grounds for modifying the Morrow et al. reference to derive the present invention. Hence, it is respectfully submitted that the Morrow et al. reference does not provide a basis for a *prima facie* obviousness rejection.

2. Applicant sets forth a basis which rebuts the prima facie obviousness rejection.

The outstanding Office Action states that the presently pending method claims, which fall within the scope of the prior art method and composition, would have been *prima facie* obvious from the prior art disclosure to a person of ordinary skill in the art at the time the invention was made because in the absence of sufficient evidence to the contrary, applicants claims are directed to optimization of an "art recognized variable" which is well within the purview of one of ordinary skill in the art. If, *arguendo*, it is admitted that the Office Action sets forth a *prima facie* obviousness rejection, applicants set forth herein factual evidence which rebuts that *prima facie* obviousness rejection. More specifically, applicants have determined that urinary levels of isoprostanes do not correlate with

circulating levels in plasma or the urination secretion of isometabolites that applicants have recently identified. Moreover, applicants have discovered that the inventive compounds claimed herein are initially formed esterified to phospholipids and then released intact, in free form. If these compounds were not released intact and unaltered from phospholipids, they would never be detected even if one were looking for them. Further, applicants have discovered that in urine, as much as 50% of these compounds are not in a free detectable form but are conjugated to glucuronic acid. Nobody skilled in the art in the field would have predicted this because conjugation and prostaglandins with glucuronic acid has not been found to occur to any appreciable extent. The result of this is that applicants would have never been able to detect these compounds, again even if applicants would have been looking for them.

Also related is the fact that there was no assurance that measuring the subject compounds would be found to be an accurate approach to assess oxidative stress status *in vivo*. Applicants have found that the levels of free compounds in urine do not correlate with levels in the circulation. This is significant factual evidence of the unobviousness of the present invention in view of the teaching of Morrow et al.

More specifically, the free compounds in urine do not correlate with the levels of the free compounds in circulation because the compounds in urine derive, in significant part, from local formation of the compounds in the kidney. The ramification of this is that applicants could easily have chosen to assess the reliability of measuring these

compounds in urine as a index of total body oxidative stress. This would have been rational to one skilled in the art because these compounds are not generated artifactually in urine by autoxidation during storage due to the fact that the substrate, arachidonic acid, is present in urine in only trivial quantities, unlike in plasma. Thus, measuring these compounds in urine would completely circumvent the potential artifact of these compounds by autoxidation as can occur in other biological fluids such as plasma. Had applicants chosen to do this, as others skilled in the art would have, applicants would have come away with data that would suggest that measuring isoprostanes does not in fact provide a reliable index of oxidative stress status *in vivo* in humans. Hence, those skilled in the art would not have been led to the present invention but rather, in exactly the opposite direction.

As a factual basis for the above, applicants attach hereto a copy of an article recently published in Biophysica Acta 1345 (1997) 121-135. The article is authored by applicants. The significant data is set forth on page 128 of the article.

Referring to the article, and by way of background, it is well accepted that measuring urinary metabolites is one of the most reliable approaches to assess total body production of prostaglandins. This applies to the prostanglandin-like compounds of the present invention (isoprostanes). This is because measuring a metabolite circumvents any artifactual generation of the parent compound, such as by autoxidation *in vivo* and the level of excretion of metabolites represents total body endogenous production because the metabolism of these compounds occur primarily in the liver, lung, etc. and not the kidney.

Thus, the fact that the level of excretion of the urinary metabolites very highly correlates very highly with the levels of the unmetabolized compounds of the circulation, indicates that either of these provides a reliable index of total body endogenous production of isoprostanes. However, the fact that the excretion of the metabolites does not correlate with levels of unmetabolized isoprostanes in urine indicates that had applicants initially chosen to measure unmetabolized isoprostanes in urine such as one skilled in the art would have done, to assess oxidative injury in the body, the results would not have looked promising. This is because the levels of unmetabolized compounds in urine are probably primarily derived from production of these compounds in the kidney rather than from filtration from the circulation as discussed above.

Hence, it must be concluded that the results obtained by the present invention were not expected results that one would have been lead to by the disclosure of the Morrow et al. reference, but rather the present invention provides unexpected results that certainly would have been missed by one skilled in the art performing experiments due to any implicit motivation of the Morrow et al. reference and the *in vitro* data set forth therein. The present invention was not achieved by producing expected benefits by employing a known method, but rather was achieved by obtaining unexpected results through unexpected routes. That is, the data present herein, in the form of the attached article, shows that had one taken the normal route of investigation chosen by those skilled in the art, one would have come out with negative data, thereby concluding that the present invention is not feasible. Such data provides a basis which certainly rebuts the outstanding *prima facie* obviousness rejection and

moreover provides evidence of patentability. Hence, it is respectfully submitted that generic independent claim 1 is patentable over the prior art.

It should also be noted that no mention is made of the compounds set forth in pending independent claim 9 nor is there any mention of the Office Action of the steps set forth in independent claim 14. Hence, it is respectfully submitted that all of the independent claims are patentable over the prior art.

The remaining dependent claims are all ultimately dependent upon at least one of the independent claims discussed above. No prior art reference makes up for the deficiencies of that references as applied against the independent claims as no prior art references discloses the characterizing features of the independent claims as discussed above.

It is respectfully requested the present amendment be entered in order to place the application in condition for allowance or at least in better condition for appeal. The application is in condition for allowance as the presently submitted amendment provides data which rebuts the previously set forth *prima facie* obviousness rejection. The application is at least in better condition for appeal as the present amendment amended the claims to simplify the issues on appeal, reduce the number of claims, and more clearly set forth the inventive features of the invention. Furthermore, the amendment sets forth no new issues and requires no further searching. The amendment could not have been made earlier as it addresses issues

first presented in the outstanding Office Action. Hence, it is respectfully requested that the present amendment be entered.

In conclusion, it is respectfully requested that the present amendment be entered in order to place the application in condition for allowance, which allowance is respectfully requested.

Respectfully submitted,

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